

DATA EVALUATION RECORD

1. **CHEMICAL:** Bromoxynil Octanoate.
Shaughnessey No. 035302.
2. **TEST MATERIAL:** 1) Nonradiolabeled Bromoxynil octanoate; CAS No. 1689-99-2; Lot No. CN-51033; 92.4% active ingredient; a brown solid. 2) ^{14}C -Bromoxynil octanoate in toluene; Lot No. 011H9209; 39.4 mCi/mmol; >98% radiopurity.
3. **STUDY TYPE:** Mollusc 96-Hour, Flow-Through Shell Deposition Study. Species Tested: Eastern Oyster (*Crassostrea virginica*).
4. **CITATION:** Dionne, E. 1992. (Bromoxynil Octanoate) - Acute Toxicity to Eastern Oyster (*Crassostrea virginica*) Under Flow-Through Conditions. SLI Report No. 92-2-4106. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by Rhone-Poulenc, Research Triangle Park, NC. EPA MRID No. 422445-01.
5. **REVIEWED BY:**

Rosemary Graham Mora, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Louis M. Rifici*
for RGM
Date: 6/25/93
6. **APPROVED BY:**

Louis M. Rifici, M.S.
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Louis M. Rifici*
6/25/93
Date: *Melissa Day* 3/23/92

Henry T. Craven, M.S.
Supervisor, EEB/EFED
USEPA

Signature: *3/23/92*
Date: *H.T. Craven* 3/23/92
7. **CONCLUSIONS:** This study is not scientifically sound and does not meet the guideline requirements for a flow-through mollusc shell deposition study. The concentration of test material in the test solutions increased substantially during the study. Therefore, the actual concentrations to which the test organisms were exposed are unknown. Based on mean measured concentrations, the 96-hour EC_{50} for eastern oysters exposed to Bromoxynil octanoate was $179 \mu\text{g a.i./l}$ and $155 \mu\text{g a.i./l}$ relative to the new shell growth of the dilution water control and solvent control oysters, respectively. Therefore, Bromoxynil octanoate is classified

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

- A. **Test Animals:** Eastern oysters (*Crassostrea virginica*) were obtained from P. Cummins Oyster Company, Pasadena, MD. Once received in the laboratory, the animals were held in wooden epoxy-painted trays with flowing seawater and examined for suitability in the test. The oysters were held for 10 days prior to test initiation and acclimated to a salinity of 31 parts per thousand (ppt) and a temperature of 20°C. The oysters were fed a supplementary algal diet of *Isochrysis galbana* and *Tetraselmis maculata*.

The oysters were of similar age and size and had a mean valve height of 36 ± 4 mm. Only 0.4% mortality was noted during the holding period at the testing laboratory.

- B. **Test System:** The test was conducted in a continuous flow serial diluter system (60% dilution factor). This system provided a flow rate of 75 ml/minute to each aquarium, providing six volume replacements/day. The glass test aquaria (60 x 30 x 30 cm) were each equipped with a 10-cm standpipe to regulate solution volume at 18 l. Circulation (flow rate of 1.75 l/minute) within each aquarium provided an even distribution of algae and test solution. The combined flow-through volume and recirculated volume of test solution equalled about 5 l/oyster/hour.

A temperature-controlled water bath was used to maintain test temperature ($20 \pm 2^\circ\text{C}$). The test was conducted under fluorescent lighting on a 16-hour light (intensity of 312 lux) and 8-hour dark photoperiod. Sudden transitions from light to dark and dark to light were avoided.

The dilution water was natural unfiltered seawater collected from Cape Cod Canal, Bourne, MA. The seawater had a pH of 7.7-7.9, and a salinity of 31 ppt.

A radiolabeled superstock solution (0.0985 mg a.i./ml) was prepared by combining the radiolabeled test material with acetone to a final volume of 100 ml, drying with nitrogen, then diluted to 100 ml with acetone. A 98 ml portion of this stock was combined

with 1545.2 mg (1427.8 mg a.i.) of non-labeled test material and diluted with acetone to 1890 ml, resulting in a final stock solution of 0.80 mg a.i./ml.

- C. **Dosage:** Ninety-six-hour flow-through acute test. Based on preliminary testing, five nominal test concentrations (52, 86, 140, 240, and 400 $\mu\text{g a.i./l}$), a dilution water control, and a solvent control (0.5 ml of acetone/l of dilution water) were used.

- D. **Design:** One day prior to test initiation, 3-5 mm of the new peripheral shell growth of each oyster was removed by grinding the shell to a blunt edge. Immediately prior to test initiation, the outer shell edge was buffed to remove any new shell deposition.

Fourteen aquaria (two aquaria/test concentration and control) were randomly positioned in the water bath. The test was initiated by impartially selecting and positioning 20 oysters in each test aquarium (40/treatment and control).

During the exposure period, the oysters were fed a supplementary algal diet of Isochrysis galbana and Tetraselmis maculata three times daily to maintain a concentration of 10^5 cells/ml in the test solutions.

At test initiation and every 24 hours thereafter, the oysters were observed for visible abnormalities and the physical characteristics of the test solutions were observed. The oysters were removed from the test containers after 96 hours and new shell growth was measured to the nearest 0.1 mm using a calibrated micrometer.

The temperature, salinity, pH, and dissolved oxygen concentration were measured daily in each aquarium. The temperature was also continuously monitored in one replicate of the control. Analytical determination of Bromoxynil octanoate was performed on each replicate of the control, solvent control, and test concentrations on days 0 and 4 using liquid scintillation counting.

- E. **Statistics:** The EC_{50} values (with 95% confidence limits) were determined by fitting untransformed and transformed data to a best fit linear regression curve based on least squares. Four linear regression curves were computed and the best fit of the untransformed or transformed data was selected based on the highest associated coefficient of determination (i.e., r^2).

3-23-93
2A

MRID No. 422445-01

DATA EVALUATION RECORD

1. **CHEMICAL:** Bromoxynil Octanoate.
Shaughnessey No. 035302.
2. **TEST MATERIAL:** 1) Nonradiolabeled Bromoxynil octanoate; CAS No. 1689-99-2; Lot No. CN-51033; 92.4% active ingredient; a brown solid. 2) ^{14}C -Bromoxynil octanoate in toluene; Lot No. 011H9209; 39.4 mCi/mmmole; >98% radiopurity.
3. **STUDY TYPE:** 72-3(b) Mollusc 96-Hour, Flow-Through Shell Deposition Study. Species Tested: Eastern Oyster (Crassostrea virginica).
4. **CITATION:** Dionne, E. 1992. (Bromoxynil Octanoate) - Acute Toxicity to Eastern Oyster (Crassostrea virginica) Under Flow-Through Conditions. SLI Report No. 92-2-4106. Prepared by Springborn Laboratories, Inc., Wareham, MA. Submitted by Rhone-Poulenc, Research Triangle Park, NC. EPA MRID No. 422445-01.
5. **REVIEWED BY:**

Michael W. Davy
Agronomist
Ecological Effects Branch

Signature: *Michael Davy*
Date: 12/3/92
6. **APPROVED BY:**

Daniel Rieder
Section Head
Ecological Effects Branch

Signature: *Daniel Rieder*
Date: 3-23-93
7. **CONCLUSIONS:** This study is scientifically sound and meets the guideline requirements for a flow-through mollusc shell deposition study. Based on mean measured concentrations, the 96-hour EC_{50} for eastern oysters exposed to Bromoxynil octanoate was 179 $\mu\text{g a.i./l}$ and 155 $\mu\text{g a.i./l}$ relative to the new shell growth of the dilution water control and solvent control oysters, respectively. Therefore, Bromoxynil octanoate is classified as highly toxic to Crassostrea virginica. The NOEC was 55 $\mu\text{g a.i./l}$ when comparing new shell growth of the treatment oysters to that of the dilution water control oysters. The NOEC could not be determined for treatment oysters compared to those of the solvent control since a significant reduction in new shell growth was observed at all levels.
8. **RECOMMENDATIONS:** N/A.

✓ Bgm
Solvent effects noted
used 155 PPB in
The D-Base
Bgm

This regression equation was then applied to calculate the EC_{50} and 95% confidence limits, using the method of inverse prediction (Sokal and Rohlf, 1969). An SLI computer program was used to assist in the calculation.

Williams' test was used to determine the NOEC. There was no significant difference between the control and solvent control, therefore the control data were pooled for analysis.

12. **REPORTED RESULTS:** Mean measured concentrations were 21, 38, 55, 100, and 170 $\mu\text{g a.i./l}$ (Table 2, attached). The mean measured concentrations averaged 42% of nominal concentrations. "The increase in measured concentrations after 96 hours of exposure is attributed to the limited water solubility of Bromoxynil in seawater and adsorption, at varying rates, to the high density of algae inherent in oyster tests. The unstable nature of Bromoxynil in water has been widely observed and documented at SLI. Alteration or modification of the test system or techniques could not be reasonably expected to significantly change this behavior."

No mortality was observed in any treatment group or the controls at test termination. The new shell growth data of the control oysters were pooled for comparison to the treatment oysters (Tables 3 and 4, attached). Based on new shell growth reduction of treatment oysters compared to the new shell growth of the pooled control oysters, the EC_{50} (95% confidence interval) was 170 (110-260) $\mu\text{g a.i./l}$ mean measured concentration. The slope of the concentration-response curve was 1.7. The NOEC was 55 $\mu\text{g a.i./l}$.

During the test period, the pH was 7.7-7.9, the dissolved oxygen concentration was 6.9-8.1 mg/l, the temperature was 18-21°C, and the salinity was 31 ppt.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** "Based on these data and the criteria established by U.S. EPA (1985), Bromoxynil octanoate would be classified as highly toxic to Eastern oysters."

A Good Laboratory Practice Compliance Statement and a Quality Assurance Statement were included in the report, indicating that the study was conducted in accordance with the EPA Good Laboratory Practice Standards (40 CFR Part 160).

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. Test Procedure:** The test procedures were generally in accordance with the SEP, except for the following:

The concentration of test material increased substantially from test initiation to test termination and levels were measured only at test initiation and test termination. However, the chemical is known to present difficulties with stability in water. EEB's policy is to use the measured mean.

In this study, the flow rate of the "recirculating" test solution was about 5 l/oyster/hour. According to protocols recommended by the SEP (APHA, 1981 and Anonymous, 1976) each oyster should receive a minimum of 5 l of "once-through" flow through test solution per hour. However this study is probably acceptable since a supplemental algal diet was added and control oysters met the minimum new shell growth requirement (2 mm).

- B. Statistical Analysis:** The reviewer used EPA's Toxanal computer program to calculate the 96-hour EC_{50} value (printouts, attached). Based on mean measured concentrations, the EC_{50} was 179 $\mu\text{g a.i./l}$ with a 95% confidence interval of 154-220 $\mu\text{g a.i./l}$ relative to the dilution water control, and 155 $\mu\text{g a.i./l}$ with a 95% confidence interval of 116-249 $\mu\text{g a.i./l}$ relative to the solvent control. The author of this study compared the concentrations to the pooled control.

The NOEC was 55 $\mu\text{g a.i./l}$ (Williams' test; printouts attached) when comparing new shell growth of the treatment oysters to that of the dilution water control oysters. The NOEC could not be determined for treatment oysters compared to those of the solvent control since a reduction in new shell growth was observed at all levels.

- C. Discussion/Results:** This study is scientifically sound and meets the guideline requirements for a 96-hour flow-through mollusc shell deposition acute toxicity test. The measured test concentrations increased substantially during the study period. Final replicate concentrations ranged from 144 to 267% of initial replicate concentrations. Since the author was aware of the unstable nature of this test material, measures should have been taken to monitor test concentrations on a daily basis to obtain mean measured concentrations

more representative of the actual exposure concentrations.

Based on mean measured concentrations, the 96-hour EC_{50} values were 179 and 155 $\mu\text{g a.i./l}$ relative to the new shell growth of the dilution water control and solvent control oysters, respectively. Therefore, Bromoxynil octanoate is classified as highly toxic to Crassostrea virginica. The NOEC was 55 $\mu\text{g a.i./l}$ when comparing new shell growth of the treatment oysters to that of the dilution water control oysters. The NOEC could not be determined for treatment oysters compared to those of the solvent control since a reduction in new shell growth was observed at all levels.

D. Adequacy of the Study:

- (1) Classification: Core
- (2) Rationale: N/A
- (3) Repairability: N/A.

15. COMPLETION OF ONE-LINER: Yes, June 3, 1991.